DNA Sample

↓

PCR 1
p53 exons

⟵ 1. *Taq* polymerase, hot start.
        Multiplex exon amplification.

↓ 2. Dilute 1/20.

LDR 1

⟵ 3. *Taq* Ligase, hot start.
        Allele specific multiplex
        linear amplification.

4a. Capillary electrophoresis or
     gel electrophoresis and
     fluorescent quantification.

4b. Capture on addressable array
     and fluorescent quantification.

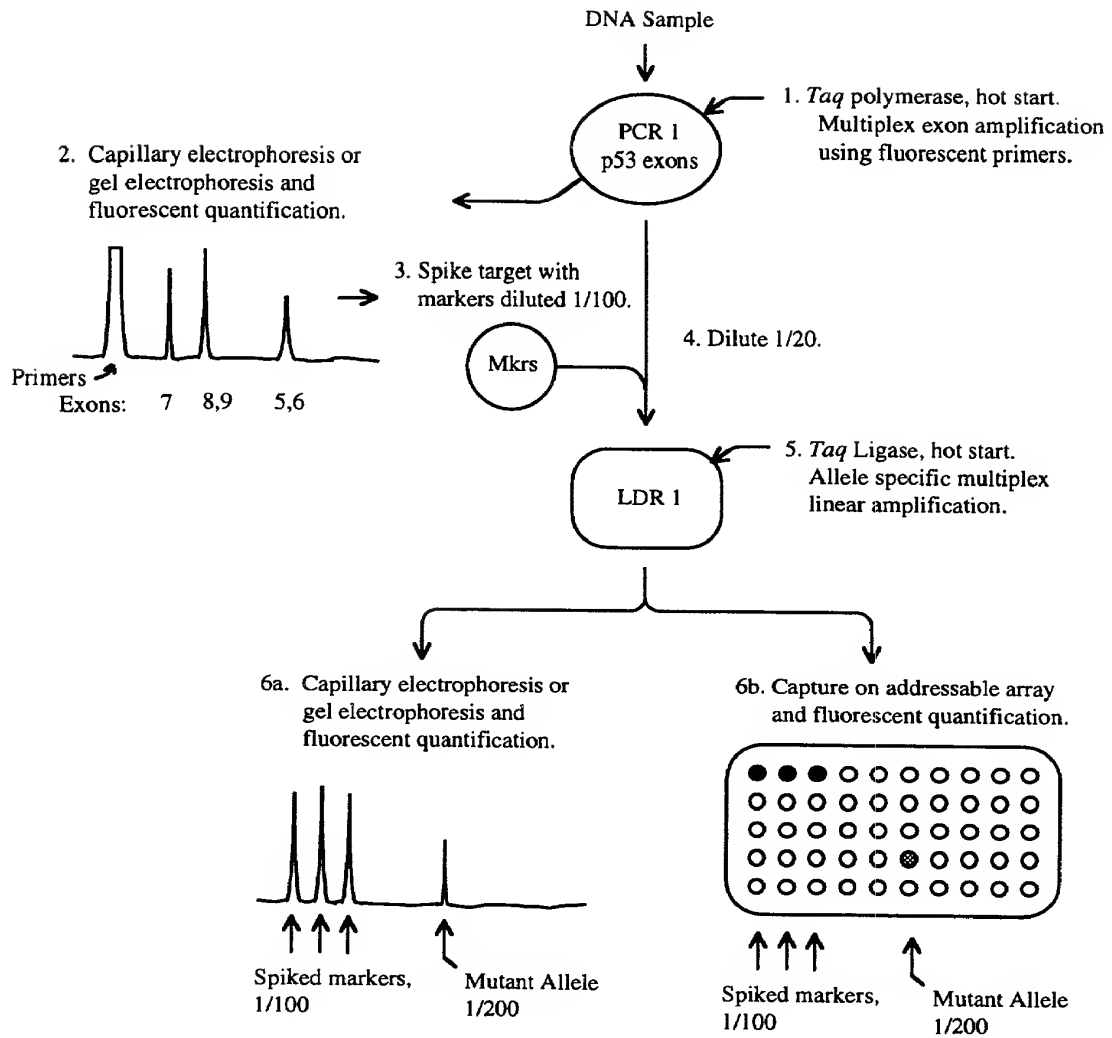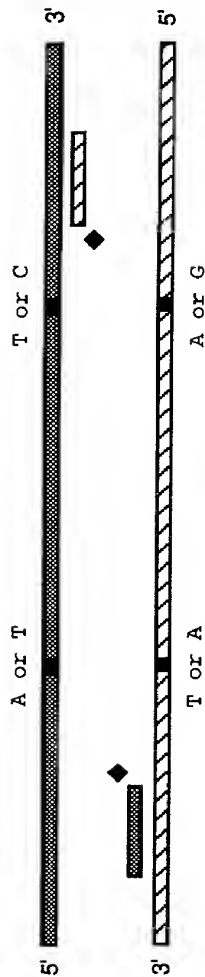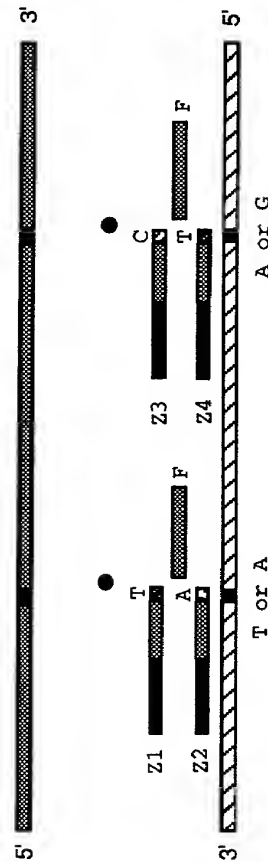Normal Allele   Mutant Allele

Normal Allele   Mutant Allele

FIG. 1

FIG. 2

PCR/LDR

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.
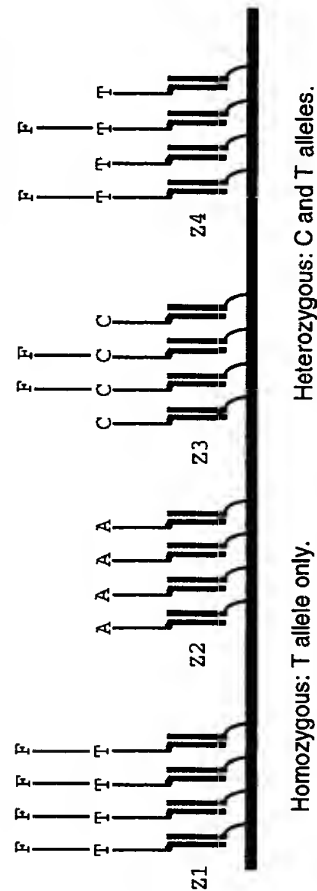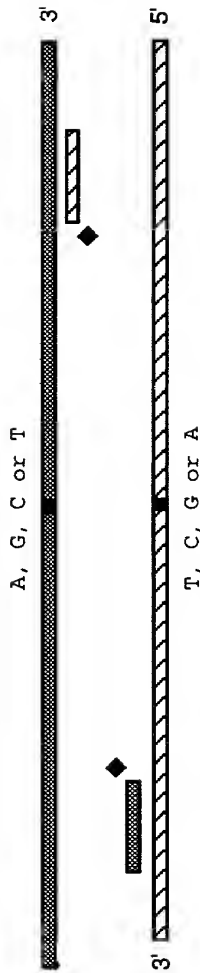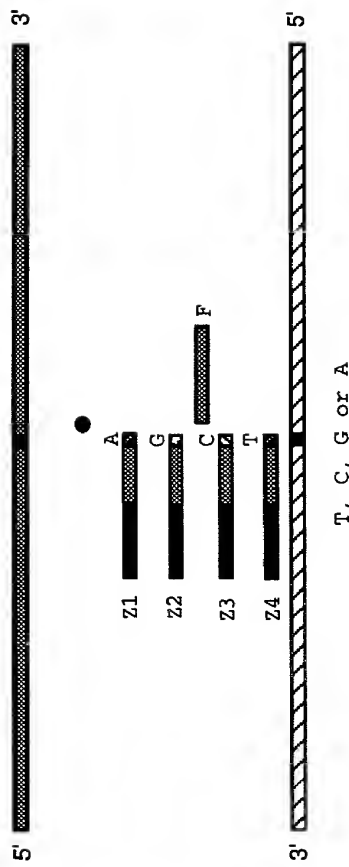
Homozygous: T allele only.   Heterozygous: C and T alleles.

FIG. 3

PCR/ LDR

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆

A, G, C or T

T, C, G or A

2. Perform LDR using allele-specific LDR primers and thermostable ligase. ●
Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

●

A z1
G z2
C z3
T z4

F

T, C, G or A

3. Capture fluorescent products on addressable array and quantify each allele.

Heterozygous: A and C alleles.

FIG. 4
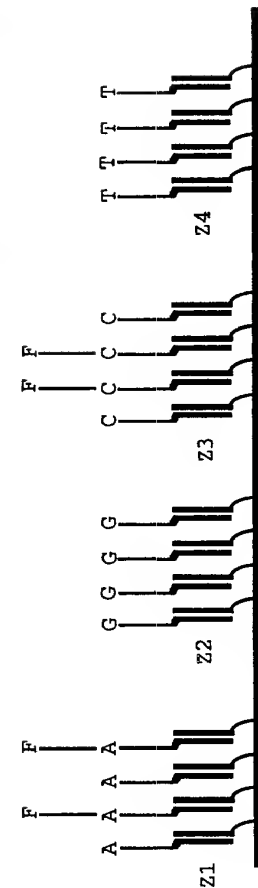
PCR/ LDR : Nearby alleles

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

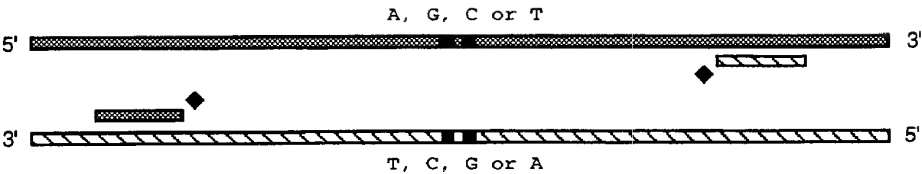3. Capture fluorescent products on addressable array and quantify each allele.
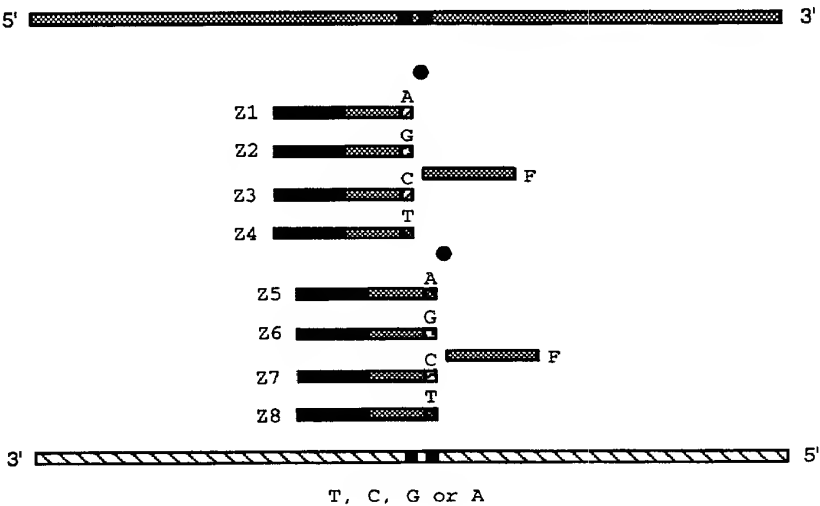

Heterozygous: A and C alleles.

Heterozygous: G,C, and T alleles.
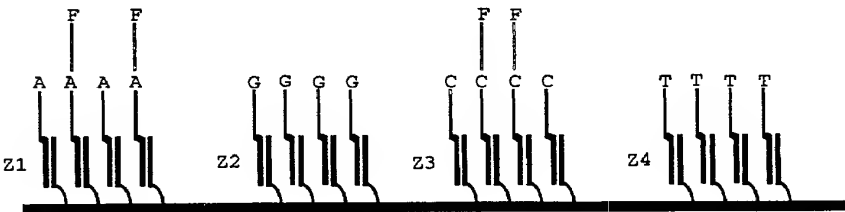
FIG. 5

PCR/ LDR : Insertions and Deletions

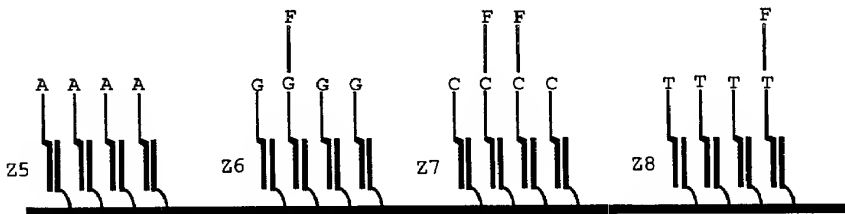1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.
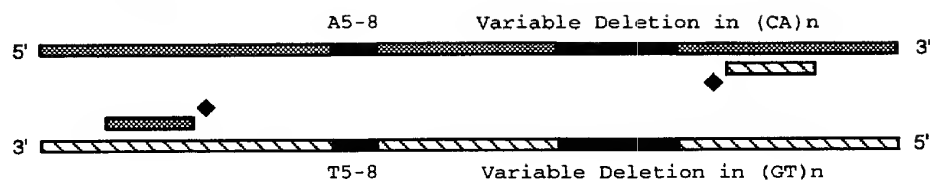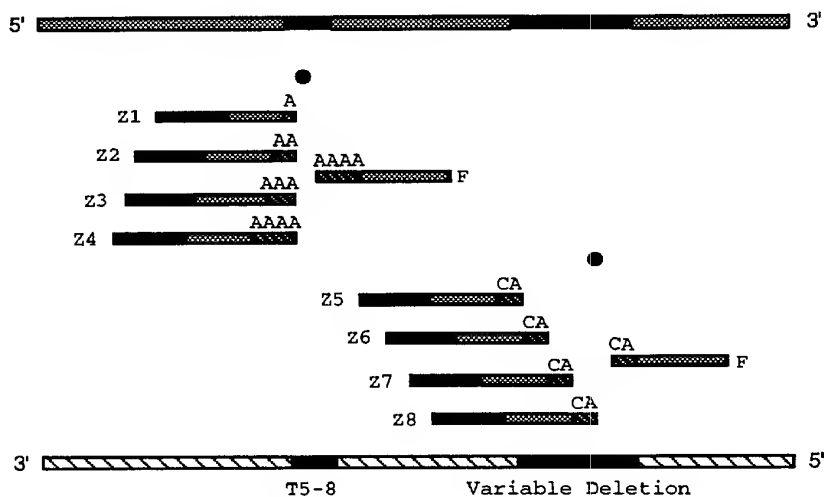

Heterozygous: A5 and A7 alleles.

Heterozygous: (CA)5 and (CA)3 alleles.

FIG. 6

PCR/ LDR : Adjacent alleles, cancer detection

Wildtype, GG

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

Wildtype, CC

2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

Z1 — A
Z2 — C
Z3 — T — F

Z4 — A
Z5 — C
Z6 — T — F

Wildtype, CC

3. Capture fluorescent products on addressable array and quantify each allele.

Z1   A A A A
Z2   C C C C
Z3   T T T T

Z4   A A A A (F)
Z5   C C C C
Z6   T T T T

Gly to Asp mutation
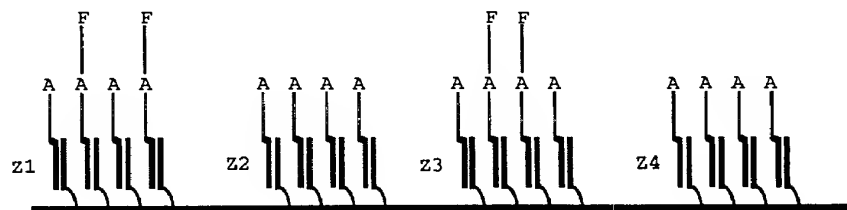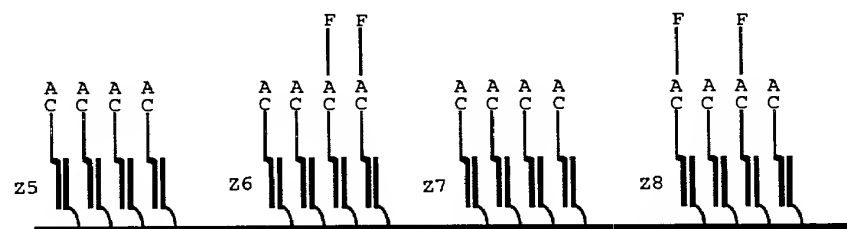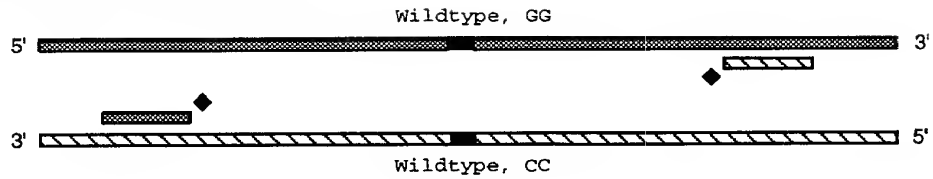
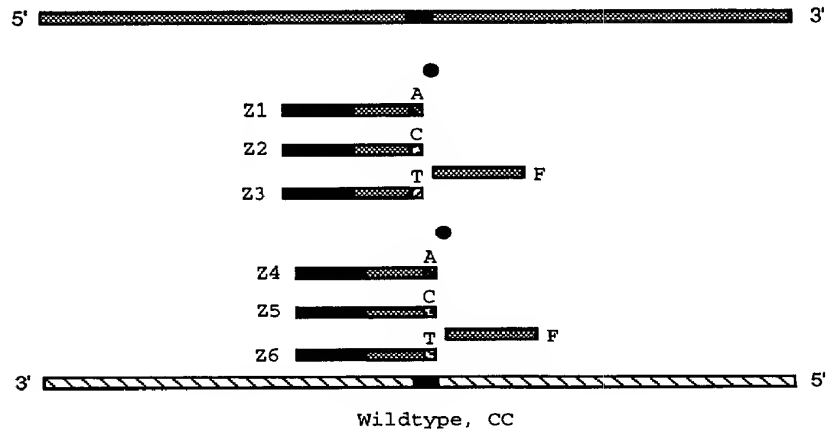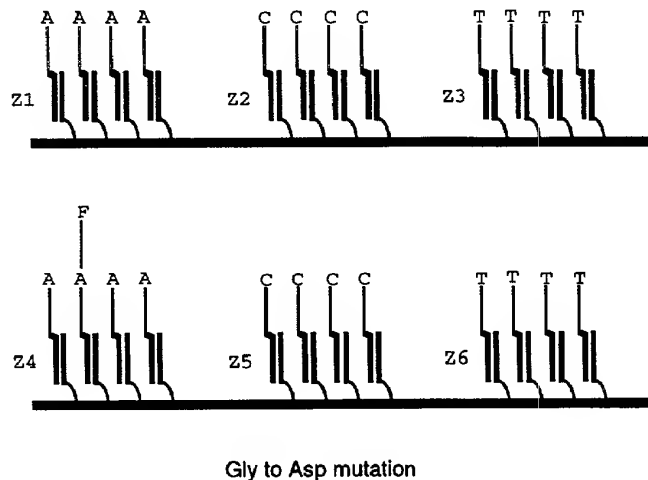*FIG. 7*

PCR/ LDR : Nearby alleles

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆

2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.

FIG. 8

PCR/ LDR : Adjacent and Nearby alleles

1. PCR amplify region(s)
   containing mutations
   using primers, dNTPs
   and *Taq* polymerase. ◆

Wildtype, GG   Wildtype, CG

Wildtype, CC   Wildtype, GC

2. Perform LDR using
   allele-specific LDR
   primers and
   thermostable ligase. ●
   Allele specific
   oligonucleotides ligate to
   common oligonucleotides
   only when there is
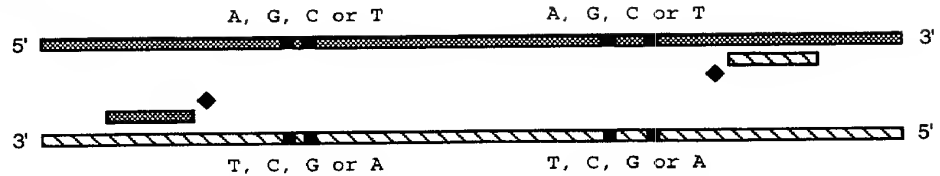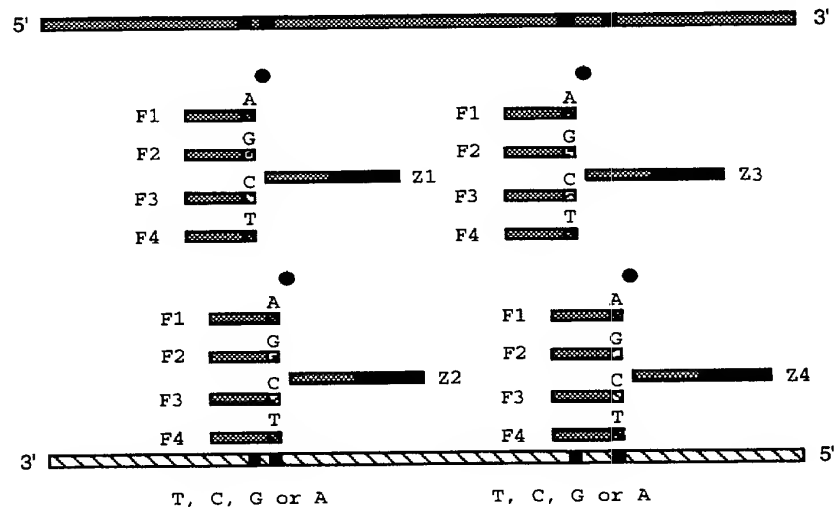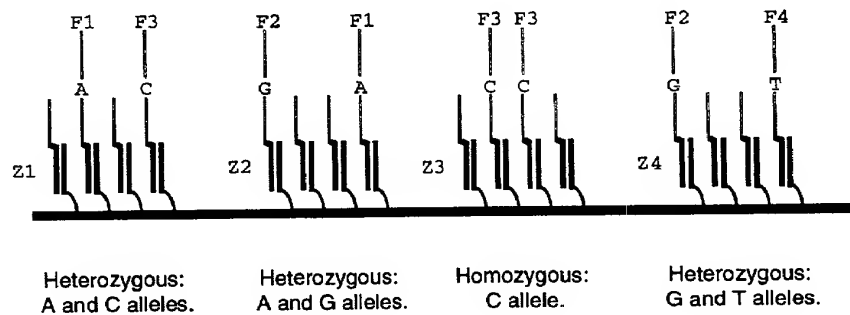   perfect complementarity
   at the junction.

Wildtype, CC   Wildtype, GC

3. Capture fluorescent
   products on addressable
   array and quantify each
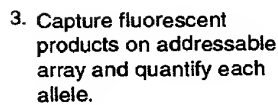   allele.

Heterozygous: Gly and Glu alleles.
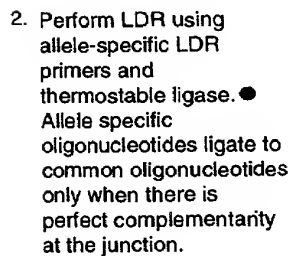
Heterozygous: Arg and Trp alleles.

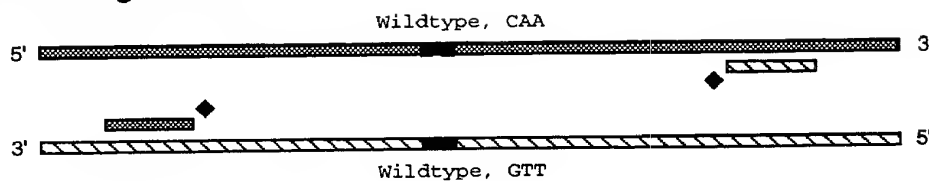*FIG. 9*

PCR/ LDR : All alleles of a single codon

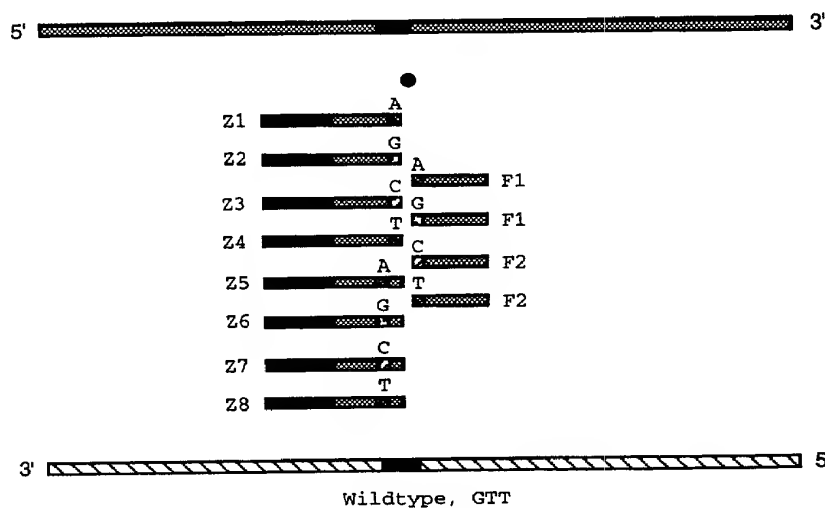1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

Wildtype, CAA

Wildtype, GTT

2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

Z1 A
Z2 G A
Z3 C F1
Z3 G
   T F1
Z4 C
   A F2
Z5 T
   G F2
Z6 G
Z7 C
Z8 T

Wildtype, GTT

3. Capture fluorescent products on addressable array and quantify each allele.

F1 F2
A   C
A A A A   G G G G   C C C C   T T T T
Z1   Z2   Z3   Z4

F1 F2
A   C
A A A A   A A A A   A A A A   A A A A
A A A A   G G G G   C C C C   T T T T
Z5   Z6   Z7   Z8
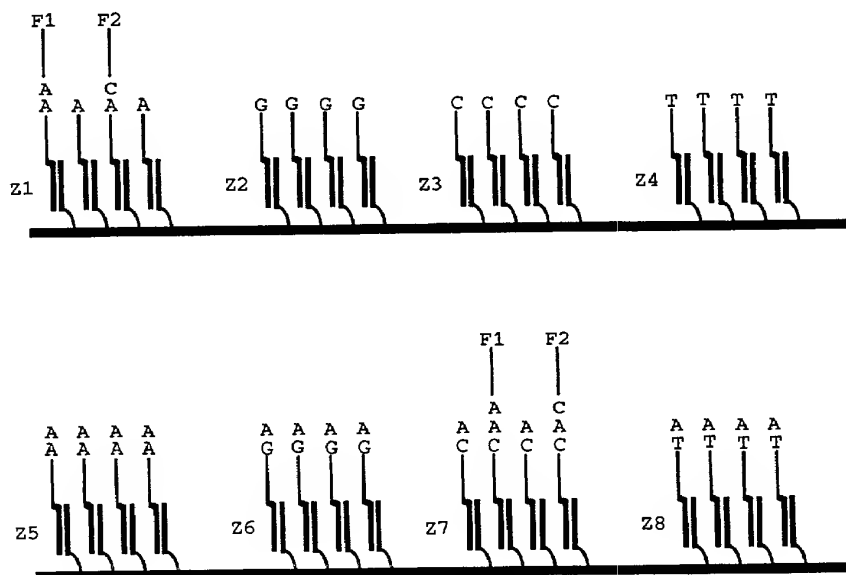
Heterozygous: Gln and His alleles.

FIG. 10

$$X \longrightarrow X^* \xrightarrow{\text{(Y)-PEG-Y}^*} \text{Y-PEG}$$

X,Y = –OH
       –CO$_2$H
       –NH$_2$
(Y) = W–NH–

X*, Y* = –O(C=O)Z
          –O(C=S)Z
          –CO$_2$H
          –(C=O)Z
          –NH$_2$
          –N=C=O
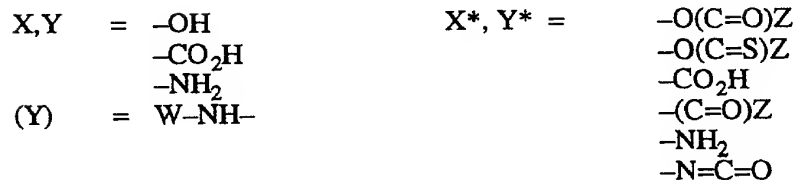
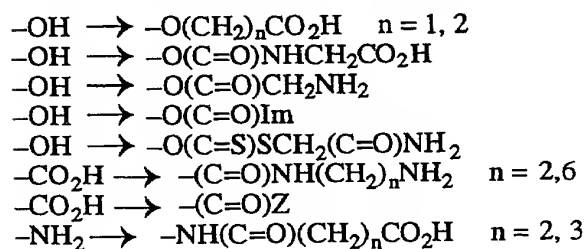W = protecting group, e.g. Boc, Fmoc
Z = activating group, e.g. imidazole (Im), p-nitrophenol (OPnp),
       hydroxysuccinimide (OSu), pentafluorophenol (OPfp)
PEG = oligo or poly(ethylene glycol), backbone (CH$_2$CH$_2$O)$_n$ $\underline{n = 6 \text{ to } 200}$
       (can also be grown by anionic polymerization with ⟨O⟩)
WSC = water soluble carbodiimide

Functional group transformations/activation (as needed), X → X*, Y → Y*

–OH → –O(CH$_2$)$_n$CO$_2$H n = 1, 2
–OH → –O(C=O)NHCH$_2$CO$_2$H
–OH → –O(C=O)CH$_2$NH$_2$
–OH → –O(C=O)Im
–OH → –O(C=S)SCH$_2$(C=O)NH$_2$
–CO$_2$H → –(C=O)NH(CH$_2$)$_n$NH$_2$ n = 2,6
–CO$_2$H → –(C=O)Z
–NH$_2$ → –NH(C=O)(CH$_2$)$_n$CO$_2$H n = 2, 3

Covalent linkage, X* + Y*

–CO$_2$H + H$_2$N– + WSC + HOSu → –(C=O)NH–
–OH + Im(C=O)Im + H$_2$N– → –O(C=O)NH–

–OH + O=C=N– → –O(C=O)NH–
–O(C=S)SCH$_2$(C=O)NH$_2$ + H$_2$N– → –O(C=S)NH–
–OH + ClCH$_2$⟨O⟩ + HO– → –OCH$_2$CH(OH)CH$_2$O–
       (+ H$_2$NH–)   (NH–)

–OH → –OCH$_2$(C=O)H + H$_2$N– + NaCNBH$_3$ → –OCH$_2$CH$_2$NH–

FIG. 11

FIG. 12A $BrCH_2-\overset{O}{\overset{\|}{C}}$—(Probe) +   HS——[Support]

pH 8

(Probe)—$\overset{O}{\overset{\|}{C}}$-$CH_2$-S—[Support]

FIG. 12B   Ac·Cys—(Probe) +   [maleimide]N—$(CH_2)_n\overset{O}{\overset{\|}{C}}$—[Support]

n = 1, 2, or 5

pH 8

$CH_3\overset{O}{\overset{\|}{C}}$-N-CH-$\overset{O}{\overset{\|}{C}}$—(Probe)
    H |
         CH_2
         |
         S—[succinimide]N—$(CH_2)_n\overset{O}{\overset{\|}{C}}$—[Support]

FIG. 12C   (Trp$_n$)—(Probe) + $HOCH_2$—[benzene ring, CH_3O top, CH_3O bottom]—$O(CH_2)_4\overset{O}{\overset{\|}{C}}$—[Support]

dilute acid

(Trp$_n$)—(Probe)

[indole ring with NH]—CH_2—[benzene ring, CH_3O, CH_3O]—$O(CH_2)_4\overset{O}{\overset{\|}{C}}$—[Support]
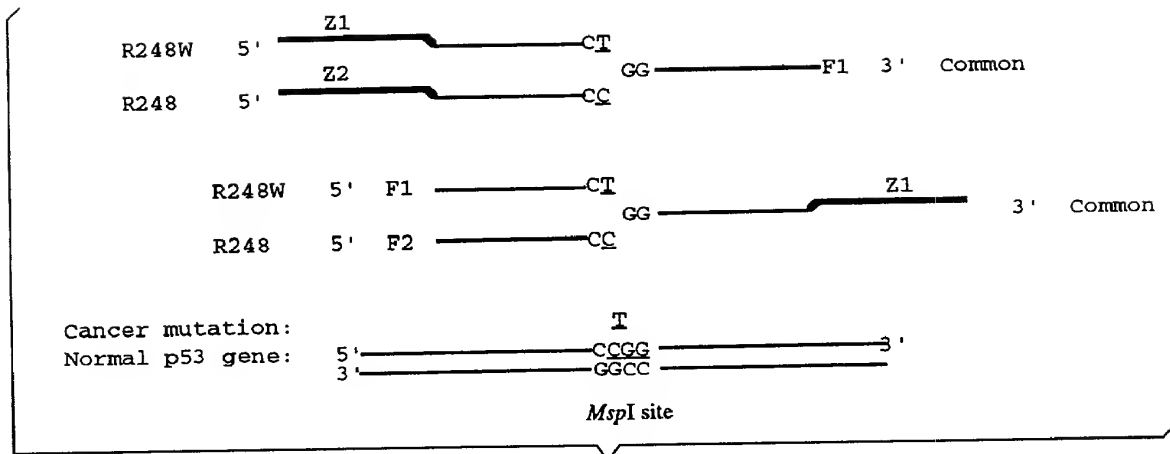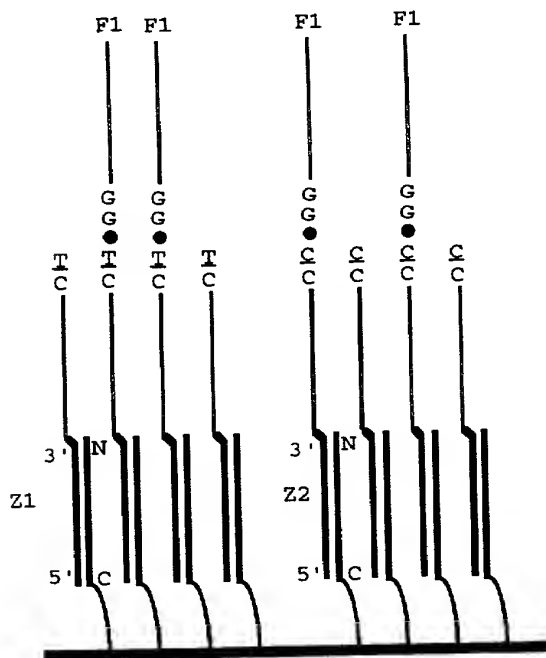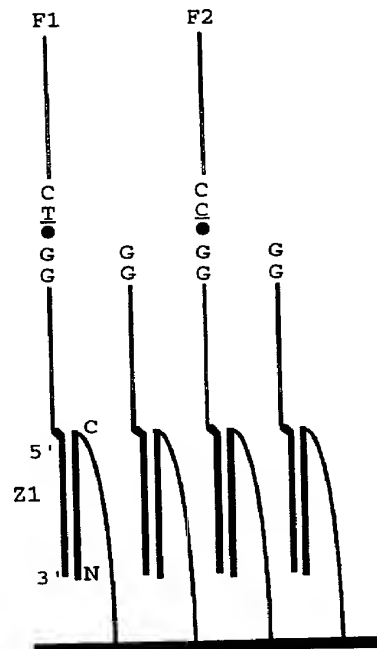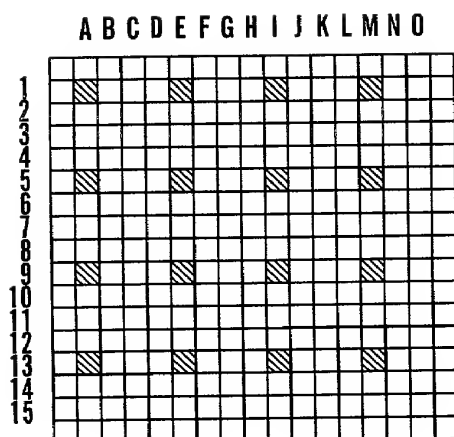
FIG. 13A

FIG. 13B

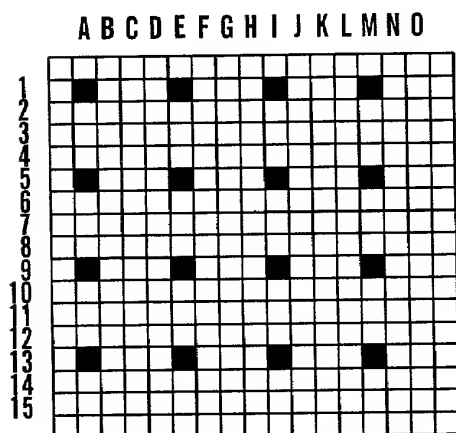

FIG. 13C

FIG. 14A
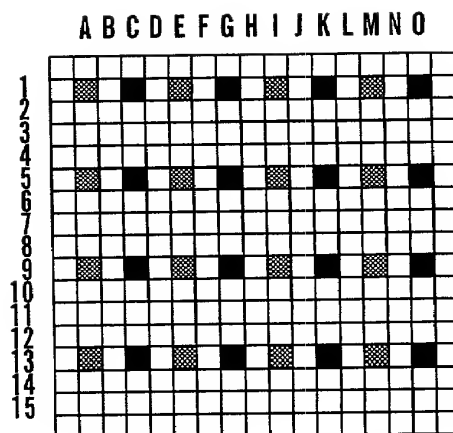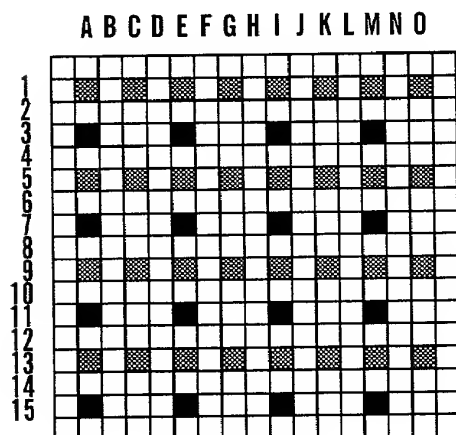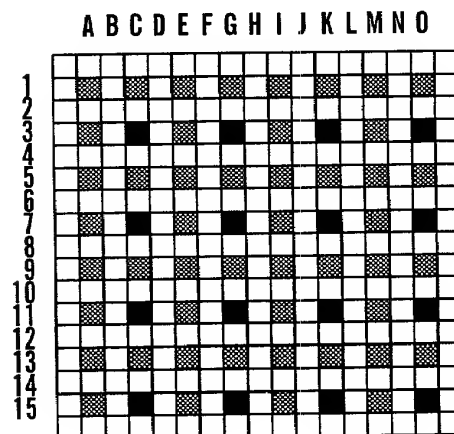

FIG. 14B

FIG. 14C

FIG. 14D

FIG. 14E
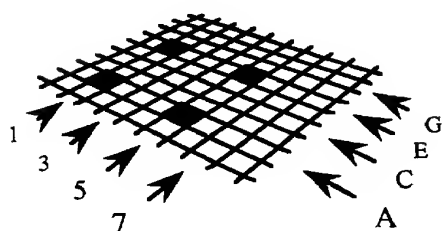
FIG. 15A

1st addition of unique 24mers.

FIG. 15B

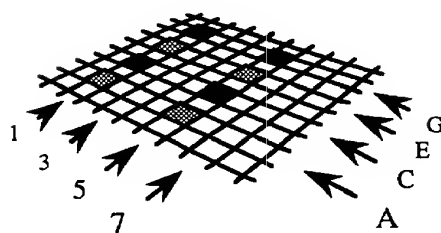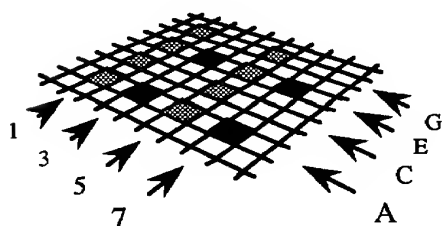2nd addition of unique 24mers.

FIG. 15C

3rd addition of unique 24mers.
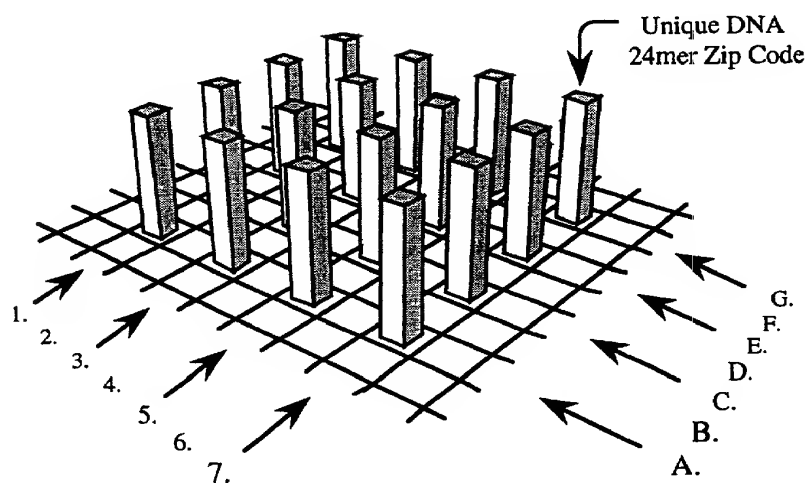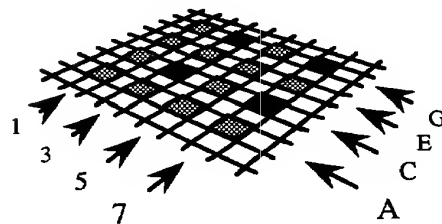

FIG. 15D
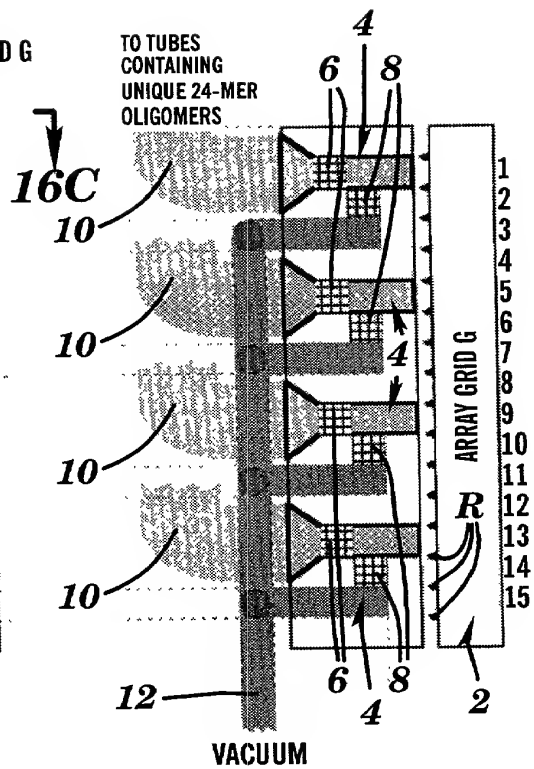
4th addition of unique 24mers.

Unique DNA
24mer Zip Code

FIG. 15E
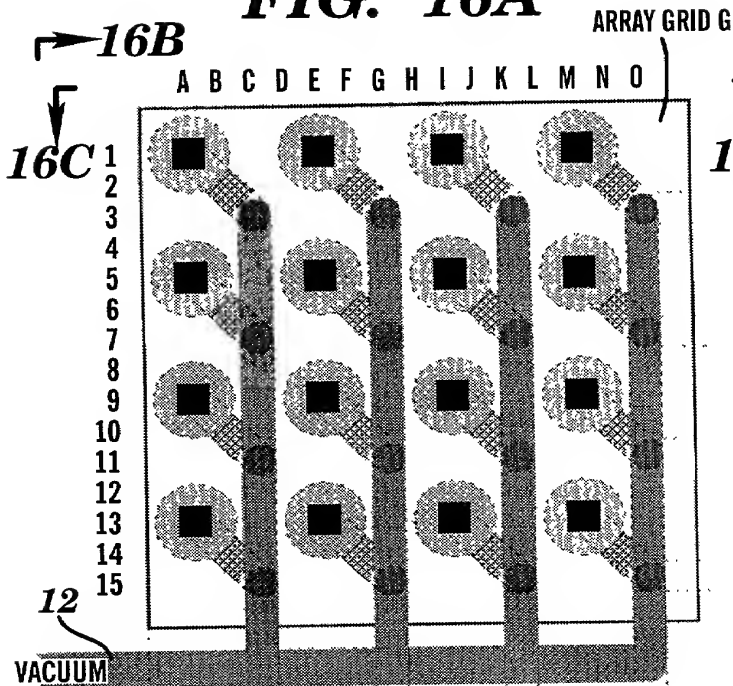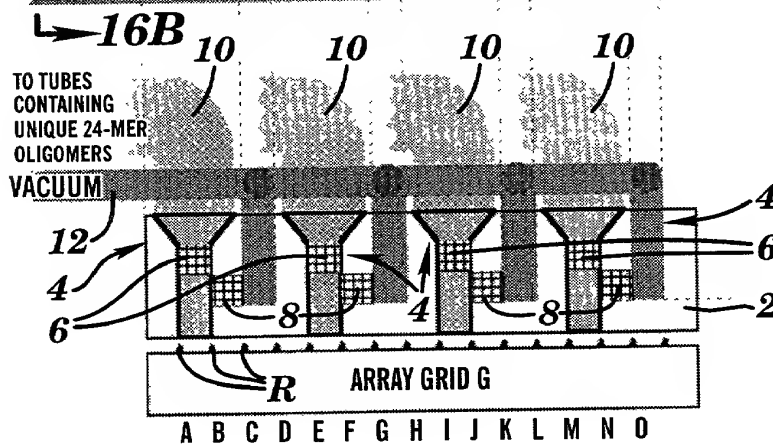
FIG. 16A

FIG. 16B

FIG. 16C

2ND TWO BASES

1ST TWO BASES

	TT	TC	TG	TA	CT	CC	CG	CA	GT	GC	GG	GA	AT	AC	AG	AA
TT							16'			23'		TTGA 6			TTAG 8	
TC			TCTG 1		30'	TCCC 3			TCGT 5							6'
TG		TGTC 2		36'			TGCG 4						TGAT 7		11'	
TA						18'		TACA 36			33'					
CT	32'		CTTG 9					CTCA 11	CTGT 13							8'
CC				CCTA 33						29'			CCAT 15			
CG	CGTT 10		12'					4'					28'			CGAA 16
CA		34'		25'			CAGC 12			CAGC 14		1'			9'	
GT					GTCT 19	24'				GTGC 22			31'			
GC	CGTT 17		14'											22'		GCAA 23
GG		20'		GGTA 18	35'							3'		GGAC 24		
GA			GATG 34			GACC 20		2'	GAGT 21							
AT						ATCG 28	7'				15'			ATAC 31		
AC		21'			ACCT 27						ACGG 29	5'			13'	
AG			AGTG 25			AGCC 35			27'		AGGA 30				19'	
AA		AATC 26					10'			17'					AAAG 32	

FIG. 17

1st Tetramer addition
(columns)

1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5

FIG. 18A

2nd Tetramer addition
(rows)

6	6	6	6	6
5	5	5	5	5
4	4	4	4	4
3	3	3	3	3
2	2	2	2	2

FIG. 18B

3rd Tetramer addition
(columns)

3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1

FIG. 18C

4th Tetramer addition
(rows)

2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5
4	4	4	4	4

FIG. 18D

5th Tetramer addition
(columns)

6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4

FIG. 18E

6th Tetramer addition
(rows)

3	3	3	3	3
2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5

FIG. 18F

Addressable array with full length PNA 24mers

1-6-3-2-6-3	2-6-4-2-1-3	3-6-5-2-2-3	4-6-6-2-3-3	5-6-1-2-4-3
1-5-3-1-6-2	2-5-4-1-1-2	3-5-5-1-2-2	4-5-6-1-3-2	5-5-1-1-4-2
1-4-3-6-6-1	2-4-4-6-1-1	3-4-5-6-2-1	4-4-6-6-3-1	5-4-1-6-4-1
1-3-3-5-6-6	2-3-4-5-1-6	3-3-5-5-2-6	4-3-6-5-3-6	5-3-1-5-4-6
1-2-3-4-6-5	2-2-4-4-1-5	3-2-5-4-2-5	4-2-6-4-3-5	5-2-1-4-4-5

FIG. 18G
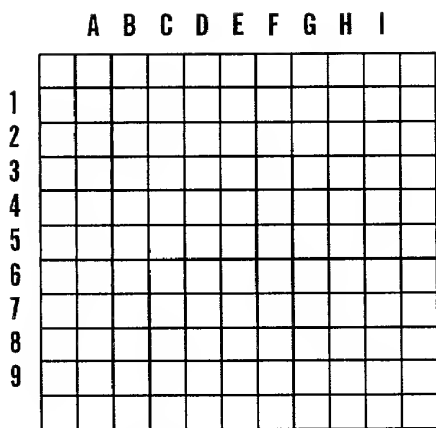
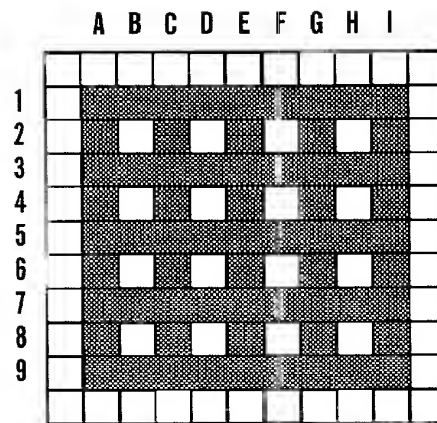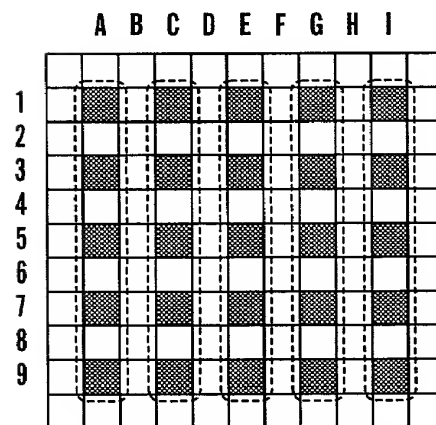**FIG. 19A**
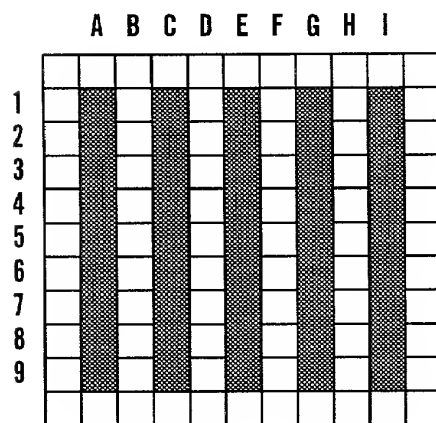
FIG. 19B

FIG. 19C
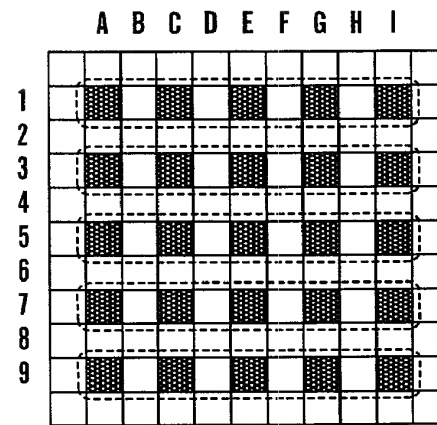

FIG. 19D

FIG. 19E

FIG. 20A

1st Tetramer additions
(columns)
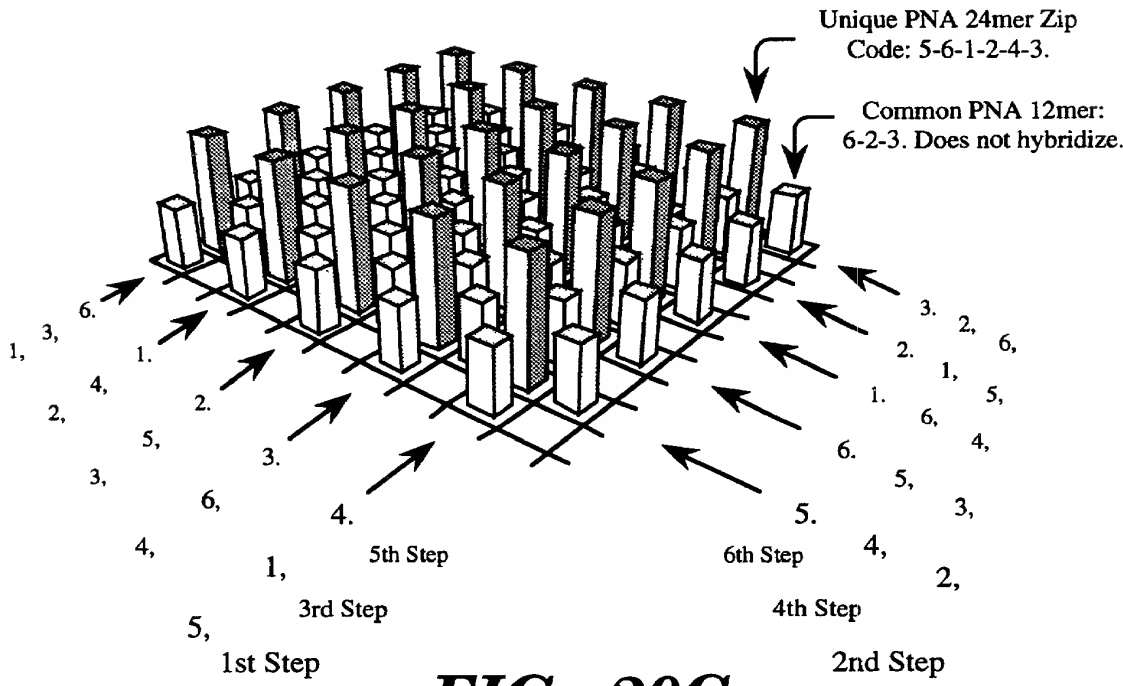

FIG. 20B

2nd Tetramer additions
(rows)

Unique PNA 24mer Zip
Code: 5-6-1-2-4-3.

Common PNA 12mer:
6-2-3. Does not hybridize.
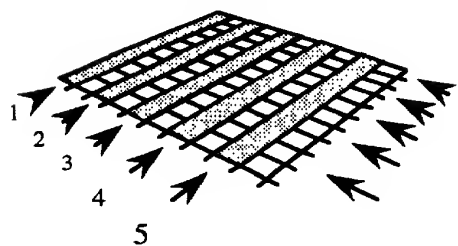
FIG. 20C
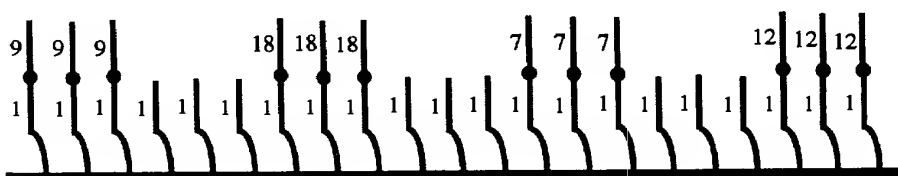
FIG. 21A

FIG. 21B

FIG. 21C

FIG. 21D

FIG. 21E

FIG. 21F

FIG. 22A

A B C D E F G H I

1 2 3 4 5 6 7 8 9

ARRAY GRID G

VACUUM

TO MANIFOLD
CONNECTED TO
TUBES CONTAINING
PNA TETRAMERS

4 4 4 4 4

6 6 6 6 6

10 10 10 10 10

8 8 8 8 8

VACUUM

2

4 4 4 4 4

R ARRAY GRID G

A B C D E F G H I

FIG. 22C

ARRAY GRID G

1 2 3 4 5 6 7 8 9

R
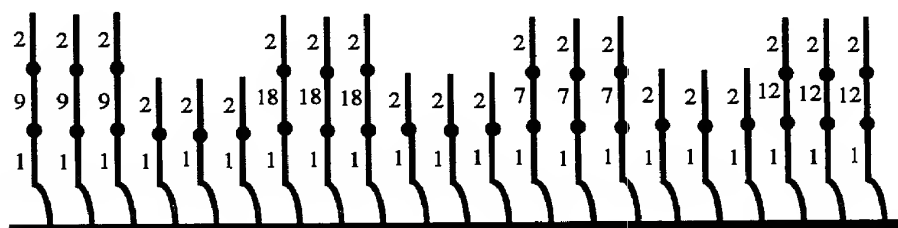
TO MANIFOLD
CONNECTED TO
TUBES CONTAINING
PNA TETRAMERS
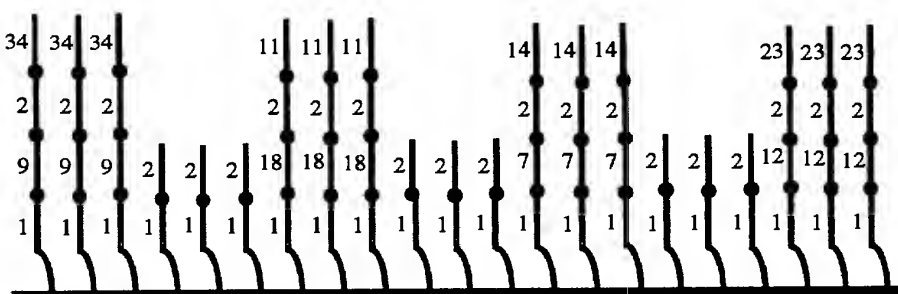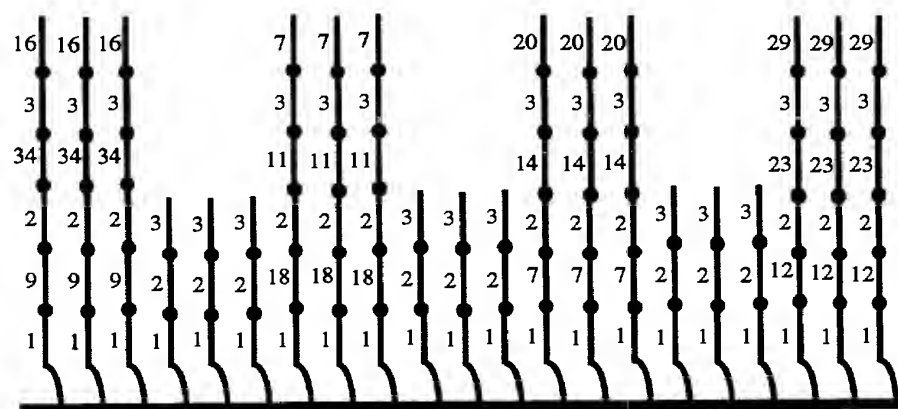
FIG. 22B

FIG. 23A

1st Tetramer additions
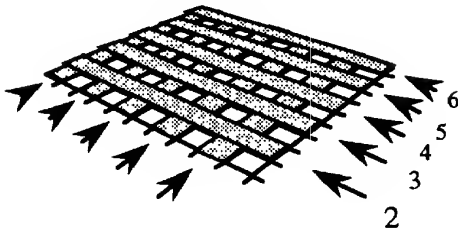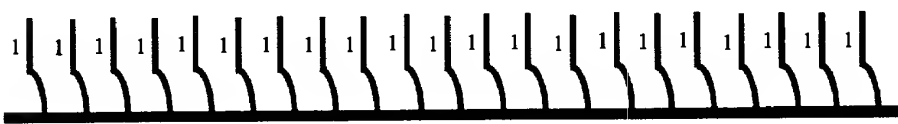(columns)

FIG. 23B

2nd Tetramer additions
(rows)

Unique PNA 24mer Zip
Code: 5-6-1-2-4-3.

FIG. 23C

FIG. 24A

ARRAY GRID G

VACUUM

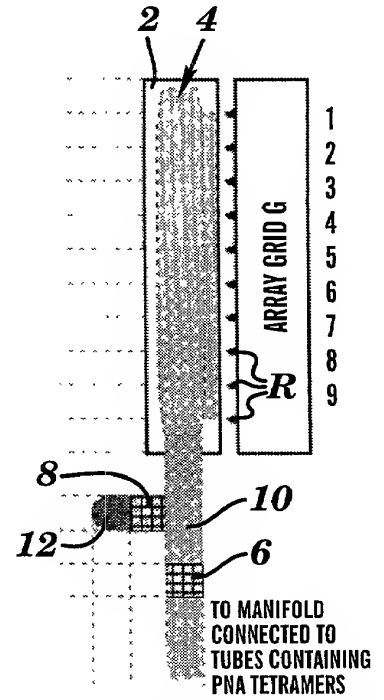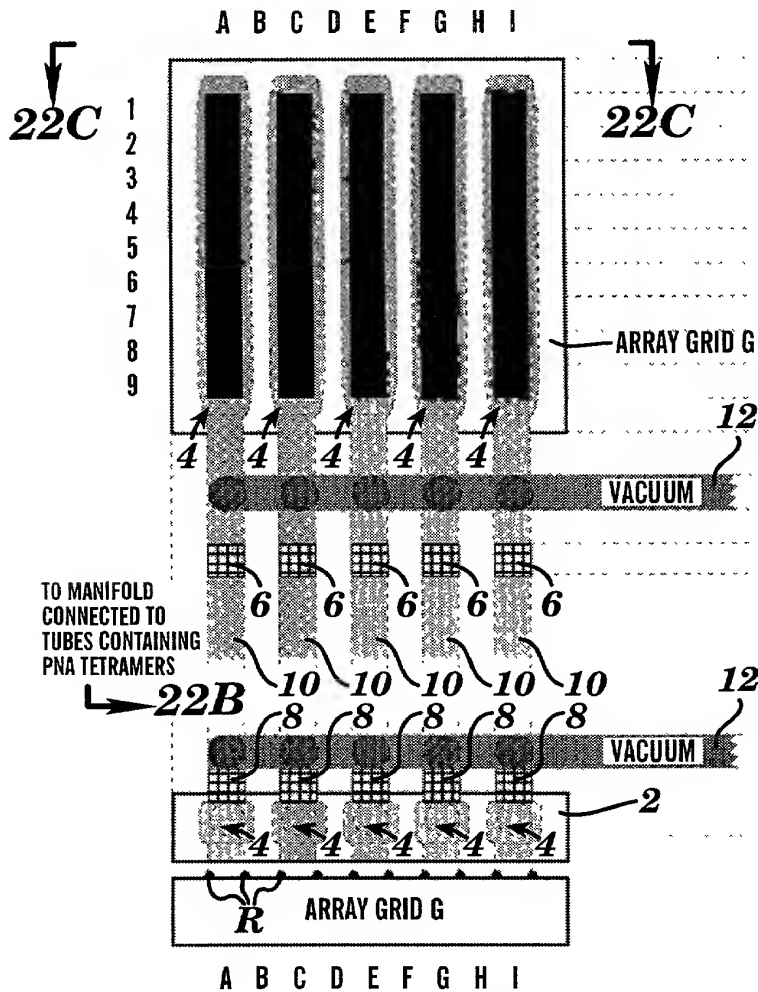TO MANIFOLD
CONNECTED TO
TUBES CONTAINING
PNA TETRAMERS

VACUUM

ARRAY GRID G

FIG. 24C

FIG. 24B

TO MANIFOLD
CONNECTED TO
TUBES CONTAINING
PNA TETRAMERS

ARRAY GRID G

VACUUM

10 *6* *14* *6* *10*

FIG. 25B

18

25C

25B *12* *25B*

8

10 6 *20 16*

PNA TETRAMER 1 CHAMBER 1

10 6 *20 16*

PNA TETRAMER 2 CHAMBER 2

10 6 *20 16*

PNA TETRAMER 3 CHAMBER 3

10 6 *20 16*

PNA TETRAMER 4 CHAMBER 4

10 6 *20 16*

PNA TETRAMER 5 CHAMBER 5

10 6

PNA TETRAMER 6 *18*

14

24

22 SOLVENT

25C

FIG. 25A VALVE BLOCK ASSEMBLY

12

8

16

16

16

16

16

18

14

24

22
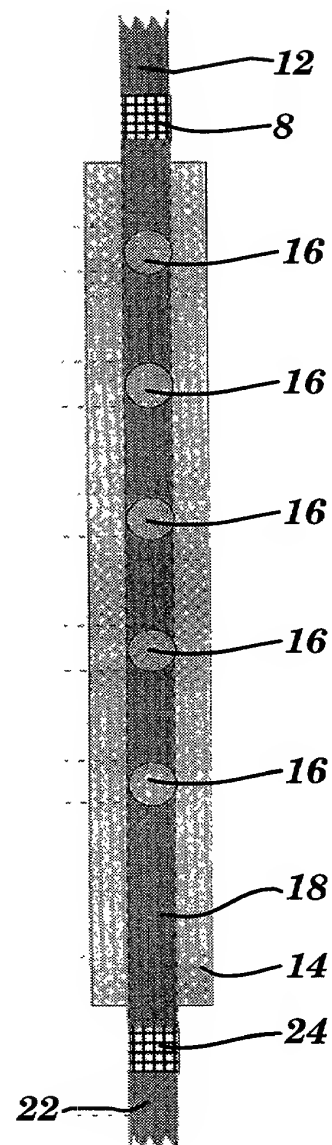
FIG. 25C

6 INPUTS AND 5 OUTPUTS

FIG. 26A

116   132   116
134

132

116   116

26A   116   134

26A   116

132   114

114A

130

26C   26C

110   110

FIG. 26B

110 106   136   110
138

138   136   106
138

106   130   136

110   136   110
134

136   110   114B
138

106   106

110   136   106   136 138
138   110

FIG. 26C

REACTION
CHAMBER

116

132

MANIFOLD
OUTPUT

ROTATING CIRCULAR
MANIOLD ASSEMBLY

6 INPUTS AND 5 OUTPUTS

MANIFOLD
INPUT   130   138   124
136

106   SOLVENT

110   108   122
REAGENT   VACUUM   112

FIG. 26D

*In Situ*PCR System 1000

sample
loading
device

hybridization chamber

pump

waste reservoir

multiple
port
system

liquid reservoirs

FIG. 27

-COOH; PROBE 12

-COOH; PROBE 14

-NH2; PROBE 12

-NH2; PROBE 14

FIG. 28

2% EGDMA

2% HDDMA

4% EGDMA

FIG. 29

1 ●
2 ●

FIG. 30

FIG. 31

H_2C
‖
$C-CH_3$
|
$O=C$
|
O
|
CH_2
|
CH_2
|
CH_2
|
Si

$+$

$$H_2C=C-\overset{\overset{\displaystyle O}{\|}}{C}-(OCH_2CH_2)_n-OH$$
|
CH_3 $n\sim 5$

AIBN
Δ or hv

OH
|
O
|
O
|
O
|
O
|
O
|
$C=O$
|
CH_3 ... $C=O$
|
$-CH_2-C-CH_2-C-$
|
$O=C$ CH_3
|
O
|
CH_2
|
CH_2
|
CH_2
|
Si

FIG. 32

H_2C
$\parallel$
$C-CH_3$
$O=C$
O
CH_2
CH_2
CH_2
Si
O O O

$CH_2\!-\!\!-\!(O\!-\!CH_2\!-\!CH_2)_\ell\!-\!O\!-\!\overset{\displaystyle O}{\overset{\|}{C}}\!-\!CH\!=\!CH_2$

$C_2H_5\!-\!C\!-\!CH_2\!-\!(O\!-\!CH_2\!-\!CH_2)_{\overline{m}}\!-\!O\!-\!\overset{\displaystyle O}{\overset{\|}{C}}\!-\!CH\!=\!CH_2$

$CH_2\!-\!\!-\!(O\!-\!CH_2\!-\!CH_2)_{\overline{n}}\!-\!O\!-\!\overset{\displaystyle O}{\overset{\|}{C}}\!-\!CH\!=\!CH_2$

$\ell + m + n \sim 14$

$H_2C\!=\!CH\!-\!\overset{\displaystyle O}{\overset{\|}{C}}\!-\!OH$

AIBN
Δ or hν

CO_2H CO_2H

FIG. 33

$$H_2C$$
$$\parallel\ \ \ \ CH_3$$
$$O=C$$
$$|$$
$$O$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$Si$$

$$CH_2\!-\!\!\!-(O\!-\!CH_2\!-\!CH_2)_{\ell}\!-\!O\!-\!\overset{\displaystyle O}{\overset{\parallel}{C}}\!-\!CH\!=\!CH_2$$

$$C_2H_5\!-\!\overset{\displaystyle }{\underset{\displaystyle }{C}}\!-\!CH_2\!-\!(O\!-\!CH_2\!-\!CH_2)_{\overline{m}}\!-\!O\!-\!\overset{\displaystyle O}{\overset{\parallel}{C}}\!-\!CH\!=\!CH_2$$

$$CH_2\!-\!\!\!-(O\!-\!CH_2\!-\!CH_2)_{\overline{n}}\!-\!O\!-\!\overset{\displaystyle O}{\overset{\parallel}{C}}\!-\!CH\!=\!CH_2$$

$$\ell + m + n \sim 14$$

$$H_2C\!=\!\overset{\displaystyle }{\underset{\displaystyle CH_3}{C}}\!-\!\overset{\displaystyle O}{\overset{\parallel}{C}}\!-\!(OCH_2CH_2)_{\overline{n}}\!-\!OH$$

$$n \sim 5$$

AIBN
Δ or hν

OH   OH

FIG. 34